ULTRASTRUCTURAL CHANGES IN THE THYMUS IN EXPERIMENTAL TUBERCULOSIS

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KEY WORDS: thymus; tissue–blood barrier; epithelial cell; lymphocyte; macrophage; experimental tuberculosis.

Many investigations of the structure of the thymus at cellular and subcellular levels have recently been published. Their results have enabled a new interpretation to be made of the function of some of the structural and cellular elements of the thymus in the light of immunologic data [5, 6, 9, 12, 14, 15].

Tuberculous infection, involving active participation of T cells, is a reaction of increased sensitivity of delayed type [1]. The interest of research workers in the study of the structure of the thymus as the central organ of immunogenesis in tuberculosis has accordingly increased considerably [2, 3, 5]. However, the ultrastructural and metabolic features of its cells in tuberculosis remain almost completely unstudied apart from a few isolated publications [7].

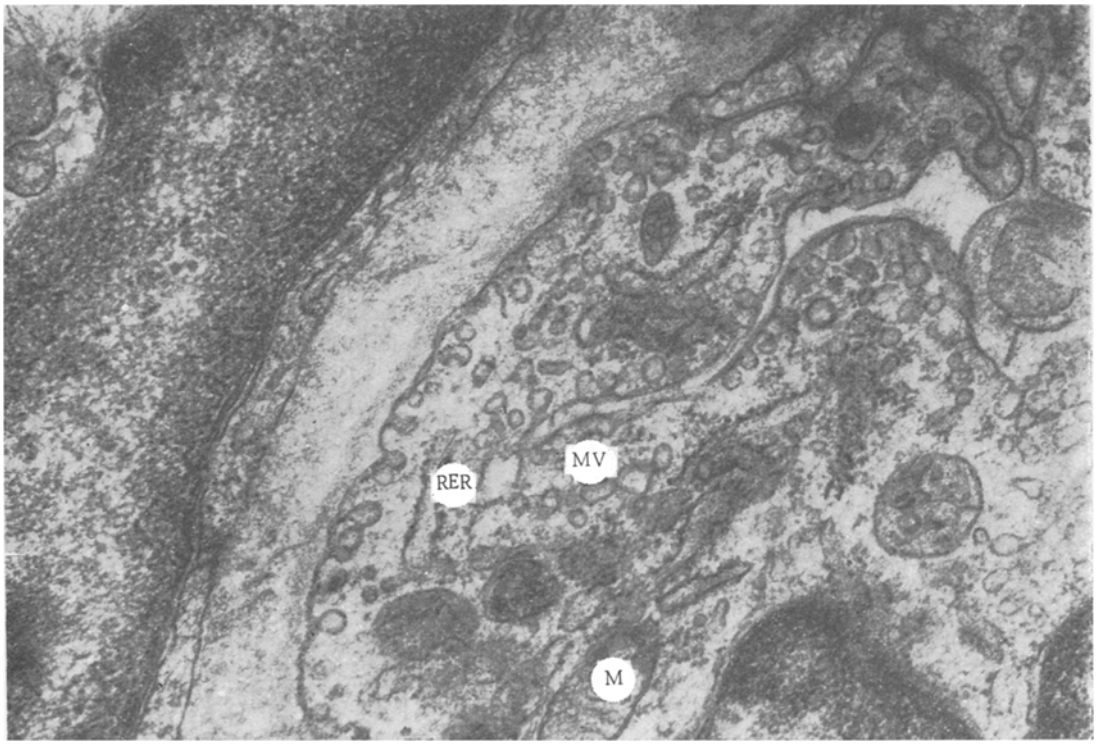

Fig. 1. Capillary endothelium in cortex of guinea pig thymus 7 days after infection, showing many micropinocytotic vesicles (MV), tubules of rough endoplasmic reticulum (RER), and mitochondria (M). 50,000 ×.

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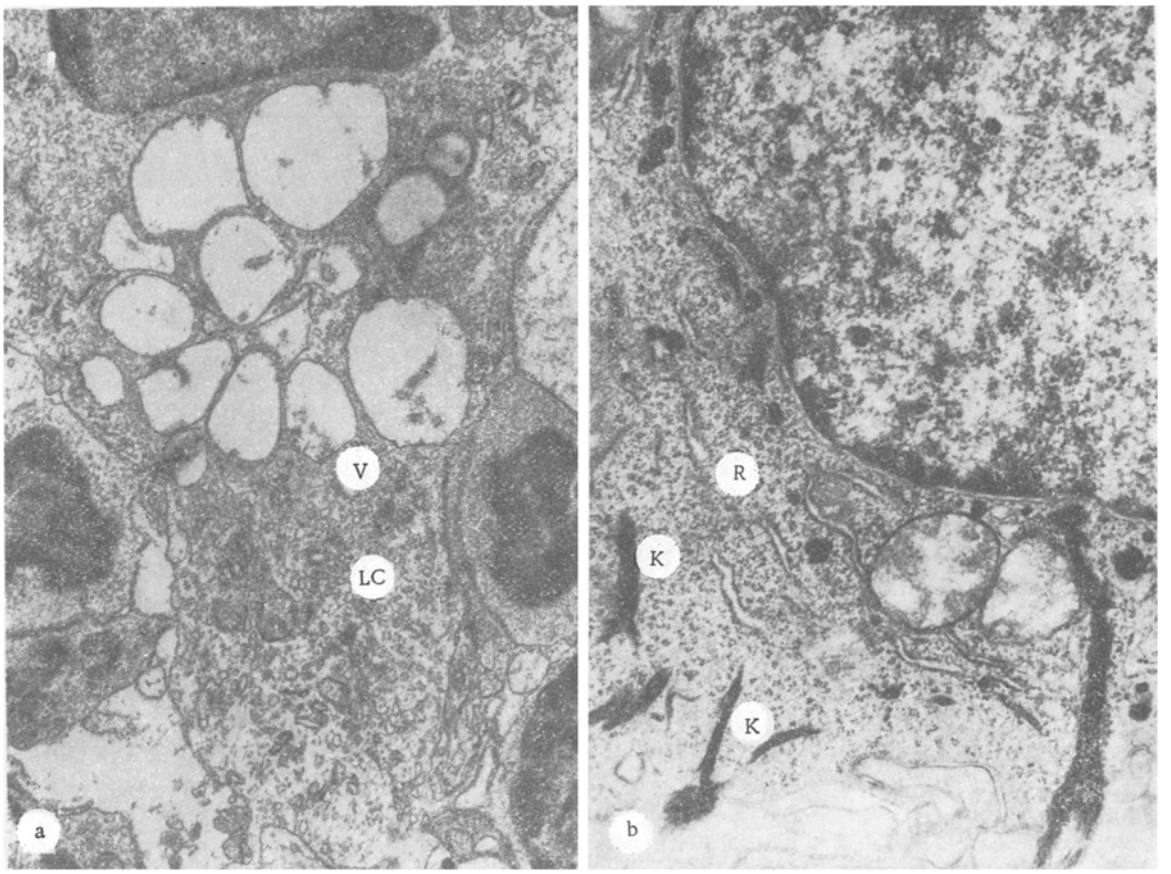

Fig. 2. Epithelial cells in medulla of thymus 7 days after infection. a) Hypertrophied cell with development of lamellar complex (LC) and formation of vacuoles (V). 9000 ×; b) peripheral cell of thymic corpuscle with keratohyalin fibrils (K) and ribosomes (R). 30,000 ×.

The aim of the present investigation was to study pathological changes developing in the thymus in experimental tuberculosis.

EXPERIMENTAL METHOD

Experiments were carried out on 73 growing guinea pigs weighing 200-250 g, infected subcutaneously with a virulent culture of *Mycobacterium tuberculosis* strain $H_{37}Rv$ in a dose of 0.0001 mg. The animals were decapitated at definite times in the course of the experiment over a period of 3 months.

Material was prepared for electron microscopy by the usual method. RNA, DNA, oxidation–reduction and hydrolytic enzymes, and nonspecific esterase were determined in histological sections. The number of cells was counted in sections stained with hematoxylin and eosin by means of an ocular grid with a magnification of 900.

EXPERIMENTAL RESULTS

The blood–thymus barrier was shown to play an important role in the development of defensive and pathological reactions arising in the thymus in experiment tuberculosis. It consists of the endothelium of the blood capillaries lining the epithelial cells and basal layers, separated by a varied amount of connective tissue. In the medullary layer pavement epithelial cells were present inconstantly between blood and lymphocytes. The tissue–blood barrier of the thymus is the place where pathological changes begin to develop in response to antigenic stimulation in animals infected with virulent strains of *M. tuberculosis* leading to disturbances of homeostasis and structural changes in the organ.

In the early stages of tuberculous inflammation (3-7 days after infection) ultrastructural changes in the components of the blood–thymus barrier were evidence of increased vascular permeability, manifested as active micropinocytosis and increased ATPase and alkaline phosphatase activity. The capillary endothelium was in an active functional state, as shown

TABLE 1. Absolute Numbers of Different Cells per Conventional Unit of Area of Guinea Pig Thymus in Experimental Tuberculosis

Cells	Time after infection				
	control	under 7 days	1 month	2 months	3 months
	Cortical layer or periphery of lobule				
Total number of cells	160,0±0,82	164,60±1,43	122,59±1,27	111,29±1,12	82,94±0,96
Small Lymphocytes	143,14±0,75	134,96±1,02	104,00±1,08	90,34±0,92	68,48±0,80
Lymphoblasts	15,90±0,06	24,76±0,34	15,31±0,14	10,76±0,09	4,61±0,09
Plasma cells	0,1±0,01	0,20±0,01	0,42±0,01	0,60±0,02	2,43±0,03
Macrophages	0,3±0,03	1,6±0,05	1,25±0,09	2,06±0,07	0,71±0,05
Cells of epithelial reticulum	0,26±0,02	1,36±0,02	0,70±0,03	2,20±0,03	0,35±0,03
Endothelial cells	0,4	1,86	0,71	4,33	4,36
	Medullary layer or center of lobule				
Total number of cells	100,0±0,41	94,45±1,12	75,41±1,12	76,51±1,27	76,86±1,12
Small Lymphocytes	79,24±0,37	58,26±0,48	51,81±0,48	51,50±0,54	60,95±0,80
Lymphoblasts	14,84±0,11	15,5±0,31	11,51±0,41	8,93±0,19	4,71±0,30
Macrophages	0,4±0,07	2,96±0,12	3,31±0,14	1,8±0,09	0,96±0,06
Epithelium	2,31±0,16	10,76±0,39	5,43±0,30	3,58±0,20	0,48±0,05
Endothelial cells	3,1±0,12	3,9±0,21	3,83±0,18	5,36±0,34	5,20±0,31
Fibroblasts	—	—	—	1,62	4,46

by the increased saturation of the cytoplasm with intracellular structures and the appearance of many micropinocytotic vesicles (Fig. 1). This enabled transcapillary exchange to be maintained at a high level and facilitated the evolution of immunologic reactions.

Between 1 and 1.5 months after infection, during the period of formation of the tuberculous granuloma and development of disseminated infection, a disturbance of vascular permeability was found with hydration of the components of the tissue–blood barrier and the onset of degenerative changes in its submicroscopic organization. As a result of accumulation of fluid the pavement epithelial cells, which were the cells most sensitive to edema, underwent destruction and died. The development of cell proliferation and stimulation of fibrillogenesis led to an increase in volume of the interstitial tissue and to a decrease in the lumen of the capillaries. This impaired transcapillary exchange and disturbed the vital functions of cells in the parenchyma of the thymus, namely lymphocytes, which underwent destructive changes. Consequently, the pavement epithelium of the cortex, forming the thin portions of the blood–thymus barrier, performs a defensive function, taking part in transcapillary exchange and delimiting the destroyed lymphocytes.

Epithelial cells of the medulla had well-marked synthetic activity, as shown by the development of organelles responsible for production and accumulation of secretion. Production of thymic hormone is known to be connected with activity of epithelial cells [11, 12], among which several types are distinguished: stellate cells, hypertrophied cells with secretory vacuoles, and cells of the thymic corpuscles [8, 10, 15].

In the early stages of the experiment an increase in the content of PAS-positive material and in the intensity of the reactions for oxidation–reduction and hydrolytic enzymes were observed in the epithelial cells. Evidence of increased secretory capacity of the epithelial cells was given by the development of a lamellar complex, especially of its vesicular and vacuolar apparatus. In the stellate cells the number of granules of lysosomal type was increased, and in the hypertrophied cells large vacuoles with floccular and granular contents (Fig. 2a) and intracellular cysts were formed, whereas in the cells of the outer layers of the thymic corpuscles fibrils of keratohyalin accumulated (Fig. 2b). Meanwhile active cell proliferation took place (Fig. 3a), accompanied by an increase in the number of lymphoblasts (Table 1), characterized by a low nucleo-cytoplasmic ratio, a well-developed ultrastructure, and an increased content of alkaline phosphatase and of oxidation–reduction enzymes. The lymphoblasts were located beneath the capsule and along the course of the postcapillary venules of the medulla. Lymphoblasts of the cortical layer are evidently young forms which are converted in the process of differentiation into mature lymphocytes, whereas lymphoblasts of the medulla are activated cells, ready to migrate into the blood stream to play their immunologic role [13, 14].

Between 7 and 14 days after infection the thymocytes underwent degenerative and destructive changes, which began in the nucleus and spread to the cytoplasm. The synthetic activity of the epithelial cells grew weaker as they matured and aged. Massive death of lymphocytes was not made good by cell proliferation or by immigration of young forms because of structural

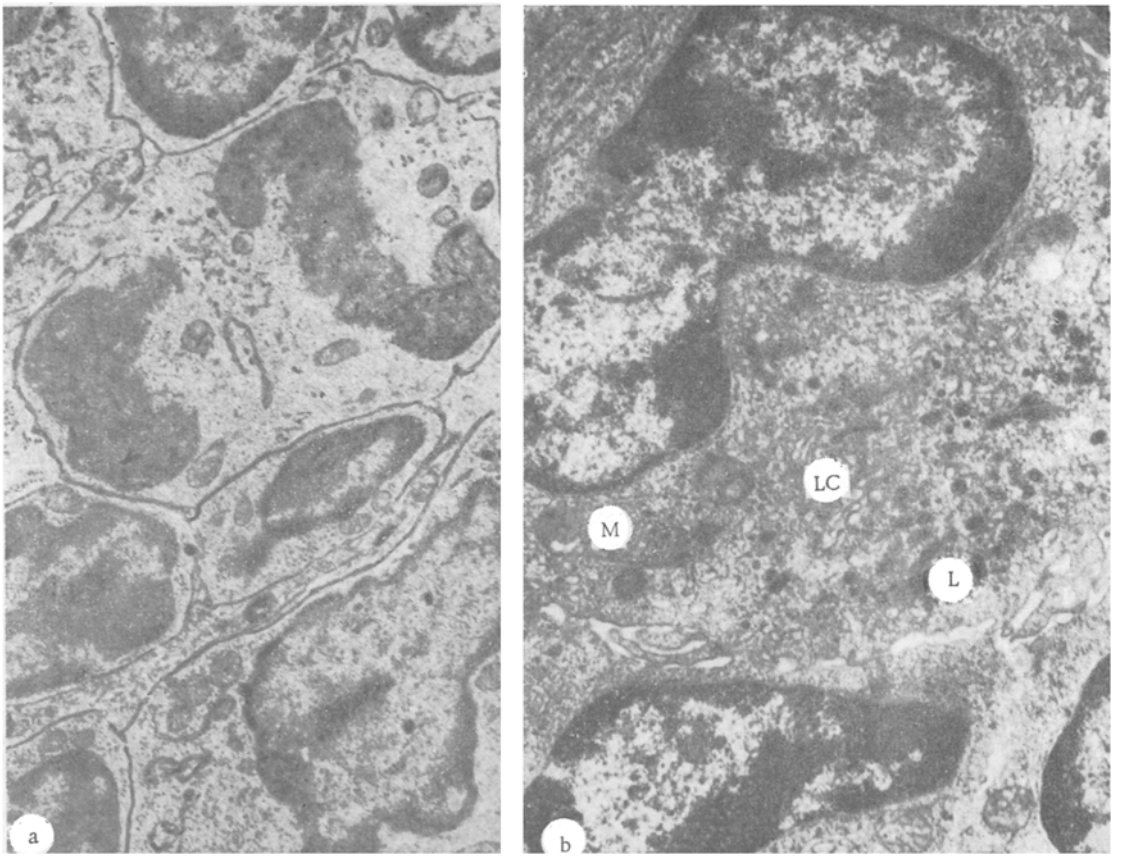

Fig. 3. Guinea pig thymus 1 month after infection. a) Mitotic division of lymphocyte. 15,000 ×; b) activated mononuclear cell: lamellar complex, lysosomes (L), mitochrondria. 17,000 ×.

and functional disturbances of the tissue–blood barrier of the thymus. The volume of the parenchyma decreased, and this was manifested as the development of accidental involution.

During the study of thymic macrophages, analysis of transitional forms (monocyte, activated mononuclear, mature macrophage) revealed their formation from mononuclear cells of monocytic origin. Precursors of macrophages were usually perivascular in distribution, in the interstitial spaces of the thymus, along the course of the postcapillary venules. They had a poorly developed cytoplasm and were poor in organelles. The activated mononuclear was characterized by the appearance of primary lysosomes (Fig. 3b) and by high motor activity of the cytoplasmic membrane. The formation of phagolysosomes indicated transformation of the mononuclear into a mature macrophage, which was usually situated in the parenchyma of the thymus among lymphocytes and was characterized by high acid phosphatase and oxidation–reduction enzyme activity. During digestion of the lymphocytes giant phagolysosomes containing cell nuclei and their fragments were formed. The number of activated mononuclear cells and mature phagocytes depended directly on the intensity of the process of thymocyte destruction.

Structural and functional changes in the thymus in experimental tuberculosis are thus phasic in character and take place in two stages. In the early stage of inflammation in tuberculosis the morphological and functional state of cells of the blood–thymus barrier is stimulated, with increased vascular permeability and migration of lymphocytes and increased synthetic activity of the medullary epithelial cells. In the period of disseminated tuberculosis cells in the parenchyma of the thymus undergo destructive changes as the result of damage to the tissue–blood barrier, leading to disturbances of homeostasis, structural changes in the organ, and the development of accidental involution. The thymic macrophages, which utilize the disintegrating lymphocytes, are monocytic and not local in origin.

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EXPERIMENTAL MODEL OF THE INITIAL STAGES OF ALIMENTARY OBESITY IN RATS

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KEY WORDS: obesity; phospholipases A; lysosomes; hepatocytes; adipocytes.

Interest in the problem of alimentary obesity has risen sharply in recent years on account of the wide distribution of this disease among the population of economically developed countries [1, 2]. The mechanisms of development of obesity and, in particular, the role of such lipolytic enzymes as the phospholipases, have not been adequately studied.

The aim of this investigation was to create an experimental model of the initial stage of alimentary obesity and, at the same time, to undertake a parallel study of the morphological and biochemical characteristics of different types of cells.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 60-70 g were used. The group of control animals received a diet containing by calorific value 20% protein, 50% carbohydrates, and 30% fat (lard and sunflower oil 1:1), together with a vitamin and salt mixture. In the diet of the experimental rats the fat content was increased to 50% (butter) and 1% cholesterol was added. The animals were allowed water and food ad lib.

After 7 months the rats were decapitated and the liver, kidneys, spleen, heart, aorta, and epididymal adipose tissue were removed for subsequent morphological and biochemical investigations. The material was fixed in 10% formalin solution and Carnoy's fluid and embedded in paraffin wax and gelatin. Sections were stained with hematoxylin–eosin, with picrofuchsin by Van Gieson's method, for lipids with Sudan III + IV according to Goldman, and with Oil red, for RNA by Brachet's method, for neutral mucopolysaccharides after Shabadash, for acid mucopolysaccharides with alcian blue after Steedman and with toluidine blue at pH 4.0 and 7.0 after preliminary treatment of some of the sections with methyl alcohol for 2 h, for calcium salts by silver nitrate after Cossa, for amyloid with Congo red, for elastic fibers with fuchselin after Weigert, and for argyrophilic fibers by Snesarev's silver impregnation method.

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